Relevance of the prostate-specific antigen (PSA) nanotest compared to the classical PSA test in the organized mass screening of prostate cancer

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Accepted for publication 12 October 2006

OBJECTIVE
To assess the reliability of a new measurement of prostate-specific antigen (PSA) using a blotting-paper assay (nanotest) compared to the standard PSA immunoassay.

SUBJECTS AND METHODS
The PSA level was measured in 205 men volunteers (median age 70 years, range 41–75) using a nanotest and a standard PSA immunoassay, collected at the same time; 30 µL of capillary blood placed on to a blotting paper were collected for the nanotest and sent by mail to the same laboratory for the two assays. The results were compared statistically using the Spearman test, the intraclass correlation coefficient and the Bland-Altman test.

RESULTS
The nanotest threshold for an abnormal PSA level was 78 pg/mL, which corresponded to a standard PSA value of 3 ng/mL, with a sensitivity of 100%. There was a significant correlation ($r = 0.98$, Spearman test; $P < 0.001$) between the nanotest and the standard PSA assay. The intraclass correlation coefficient was 0.87. The Bland-Altman test showed a good agreement between the nanotest and the standard PSA assay, but there was an increasing proportional difference with increasing PSA value.

CONCLUSION
There was a very high correlation between the nanotest and the standard PSA assay, especially for standard PSA levels of $< 5$ ng/mL. Economic and clinical studies are indicated to confirm the utility of the nanotest in organized mass screening of prostate cancer.

KEYWORDS
PSA, mass screening, prostate cancer, population surveillance

INTRODUCTION
Prostate cancer is the most diagnosed cancer in the industrialized world and the second leading cause of death from cancer in men [1]. PSA is widely recognized as an important tumour marker for prostate cancer. Since its introduction in the late 1980s, a substantially higher proportion of prostate cancer has been diagnosed in the early stages than previously [2]. Men diagnosed with prostate cancer at an early stage have the most favourable prognosis for a cure [3]. The measurement of serum PSA using a monoclonal antibody–based immunoassay allows accurate and reproducible quantification, but this procedure requires expensive equipment, test kits and skilled personal [4].

These requirements are unsuitable for a mass screening procedure. To solve the problem we developed a new method (nanotest) that permits subjects to collect blood onto blotting paper from their finger and send it by mail to the laboratory. This method reduces collecting time and the quantity of equipment required for blood collection. Thus we expect to reduce the cost of the screening procedure. Before confirming this supposition with an economic study comparing the two methods, the reliability of the nanotest must be assessed, and thus the aim of the present study was to evaluate the reliability compared to the standard PSA assay.

SUBJECTS AND METHODS
The study comprised 205 volunteers (median age 70 years, range 41–75) with no history of prostate disease, enrolled between February and July 2000 in Paris (France) by the Caisse Nationale de l’Assurance Maladie; the men were all French and Caucasian. For each man a venous blood sample and a drop of capillary blood were taken for the standard PSA and nanotest, respectively. The serum PSA values were determined simultaneously using a monoclonal antibody-based radioimmunoassay (PSA-RIACT, CIS Bio-international, Orsay, France; standard PSA) and a method developed in our laboratory, the nanotest. For the latter, a drop of capillary blood (30 µL) was taken by pricking the finger and collecting the drop on a standard blotting paper (>1 mm thick). Both samples were sent by mail to the same laboratory (Hormonology Department, Saint-Louis Hospital, Paris, France) for analysis by the two assay methods.
TABLE 1 A summary of the number (%) of subjects with a standard PSA of > or <3 ng/mL and a nanotest PSA of 78–133 pg/mL

<table>
<thead>
<tr>
<th>Nanotest PSA, pg/mL</th>
<th>Standard PSA, ng/mL</th>
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<tbody>
<tr>
<td>&lt;78</td>
<td>147 (71.7) 0</td>
</tr>
<tr>
<td>78–133</td>
<td>24 (11.7) 10 (5)</td>
</tr>
<tr>
<td>&gt;133</td>
<td>0 24 (11.7)</td>
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For the nanotest, the blood component was extracted with 100 mM Tris-HCl buffer solution (pH 7.4) including 0.1% Tween-20 for 60 min at room temperature; then 200 µL of the extracted supernatant fluid was used for the nanotest, the result being expressed in pg/mL. The PSA was assessed in the serum by an immunofluorometric assay, which requires three different monoclonal antibodies, made in the Department of Hormonology, directed against three different epitopes of the PSA molecule. The choice of the antibody was based on their ability to recognize in an equimolar manner either free PSA or PSA linked to α1-antichymotrypsin. The first antibody (PSA-2–4B2), the ‘capture antibody’ was fixed to the wells. The sera and the controls were added and allowed to bind for 2 h. A three-step reaction then allowed the detection of PSA, by adding the two remaining biotinylated monoclonal antibodies and incubating for 2 h, detecting the biotinylated antibody linked to the PSA by a streptavidin-europium complex, and revealing the europium with a solution that released the europium from the streptavidin. The fluorescence intensity at 340 nm was measured by the emission of light at 615 nm with a 1234 Delfia Fluorometer (Perkin-Elmer Wallac, Evry, France).

We compared the nanotest PSA value to standard PSA values, considering a positive standard PSA level to be >3 ng/mL, and determined the best threshold for the nanotest in this case. The values were compared statistically using the Spearman test to assess the correlation between the measurements, the intraclass correlation coefficient (ICC) to assess the agreement between them (the scale value used for the ICC being based on the reappraisal of the κ coefficient proposed by Thompson and Walter [5]), and lastly we assessed whether the difference between the two measurements was related to the magnitude of the measurement, plotting the difference against the average of the standard and new measurements [Bland and Altman diagram (6,7)].

RESULTS

Of the 205 subjects, 34 (16.7%) had a standard PSA level of >3 ng/mL; the threshold for the nanotest PSA level was thus 78–133 pg/mL to obtain 100% sensitivity and specificity, respectively. Using the threshold of 78 pg/mL, giving 100% sensitivity, 24 (11.7%) subjects had false-positive results (Table 1).

The Spearman correlation between standard PSA and the nanotest was very high (Fig. 1), with a statistically significant coefficient (r = 0.98, P < 0.001). The coefficient of variation within and between assays were, respectively, 5.2% (20 samples) and 8.4% (15 samples). The ICC was 0.87; the Bland-Altman test showed a good agreement between the nanotest and the standard PSA assay, but with an increasing proportional difference with increasing standard PSA value.

DISCUSSION

The value of PSA for detecting early prostate cancer in men with no disease symptoms was reported as early as 1989 [8,9]. As a tool for detecting early prostate cancer PSA has the highest positive predictive value [10], a reasonable cost and is widely accepted by patients [11]. However, the usual procedure for PSA assay involves taking blood from the arm, which requires a qualified nurse and specific materials and techniques; this is not suitable for mass screening. For these reasons we developed the nanotest, in which the PSA is measured from a drop of capillary blood taken by a finger prick onto blotting paper, and that can be done at home by the subject.

The great potential for this tool in the field of mass screening is the avoidance of any instrumentation and trained personnel. This might reduce the costs of potential mass screening. The most frequent self-reported reason that men gave for failure to participate in a prostate screening programme was their lack of time [12]. The drawing of capillary blood might save time for subjects and medical staff, and be less painful than drawing blood from the arm. The test could also be sent directly to the subjects by mail, to be returned the same way to the laboratory. The sample can be preserved at room temperature for up to 1 month and in a refrigerator for up to 1 year. For all these reasons greater acceptance could be expected, which is a key point in the success of a mass screening [2].

To measure the PSA in the nanotest the time-resolved fluoroimmunoassay was chosen because it is very sensitive, the minimum detectable concentration being 5 × 10^-18 mol [13]. Therefore, 30 µL of blood was sufficient for the nanotest, giving a PSA value in pg/mL of blood.

There was a very good correlation (r = 0.98; Fig. 1), comparable with that found by Watanabe et al. [14]. The relatively high ICC (0.87), which is classified as excellent agreement according to Thompson and Walter [5] (ICC >0.75), showed a good agreement, but the Bland-Altman diagram showed that the reliability of the nanotest decreased inversely with increasing standard PSA level. Figure 1 can be divided into two parts; up to 5 ng/mL of standard PSA the nanotest is reliable but above this value it seems to be less accurate. However, there were only 15 subjects of 205 (7.3%) with a standard PSA value of >5 ng/mL. A larger population would be required to confirm the decrease in reliability with increasing PSA level. However, the decrease in the reliability
of the nanotest for higher standard PSA values is probably due to a technical problem, as the Bland-Altman test gave a straight line. The inaccuracy of the nanotest for the higher PSA values was possibly linked to a saturation of the anti-PSA antibody by the high concentration of PSA molecules in the samples. Therefore, an accurate dilution of the blood sample should resolve the problem and improve the agreement for the high values of standard PSA.

However, the two large-scale randomized prostate cancer screening trials [the European Randomised Screening for Prostate Cancer in Europe and the Prostate, Lung, Colorectal and Ovary cancer trial in the USA] used, respectively, a threshold PSA level of 3 [15] and 4 ng/mL [2] for standard PSA to indicate the need for a biopsy. Furthermore, the British study of prostate cancer screening, ProtecT, has a threshold of 3 ng/mL (unpublished study of prostate cancer screening, ProtecT, the need for a biopsy. Furthermore, the British study of prostate cancer screening, ProtecT, has a threshold of 3 ng/mL (unpublished data). As the nanotest is reliable up to 5 ng/mL of standard PSA, this tool seems to be suitable for mass screening for prostate cancer. We think the most feasible use of the nanotest is for mass screening for prostate cancer.

In conclusion, there was a very strong correlation between the nanotest and the standard PSA assay. Although the reliability decreased inversely with increasing standard PSA value, the nanotest is accurate for PSA levels of <5 ng/mL. Therefore economic and clinical studies are indicated to confirm the utility of the nanotest in organized mass screening for prostate cancer.

CONFLICT OF INTEREST
None declared.

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Abbreviations: ICC, intraclass coefficient.